

A portal-arterial glucose concentration gradient as a signal for an insulin-dependent net glucose uptake in perfused rat liver

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Since in the usual perfusion of isolated rat liver via the portal vein an insulin-dependent increase of hepatic glucose uptake could not be demonstrated, the possibility was considered that hepatic glucose uptake might not be a function of the absolute concentration of this substrate but of its concentration gradient between the portal vein and the hepatic artery. Therefore a new method was established for the simultaneous perfusion of isolated rat liver via both the hepatic artery (20–35% flow) and the portal vein (80–65% flow). When glucose was offered in a concentration gradient, 9.5 mM in the portal vein and 6 mM in the hepatic artery, insulin given via both vessels caused a shift from net glucose release to uptake. This insulin-dependent shift was not observed when glucose was offered without a gradient or with an inverse gradient, 6 mM in the portal vein and 9.5 mM in the hepatic artery. Using a portal-arterial glucose gradient as a signal the liver might be able to differentiate between endogenous and exogenous glucose.

Glucose uptake Insulin (Perfused rat liver)

1. INTRODUCTION

The role of insulin in the regulation of hepatic carbohydrate metabolism is still a matter of debate. In isolated liver preparations such as cell cultures and liver perfusions many studies failed to demonstrate an effect of insulin on glucose balance, i.e. uptake or output [1–5]. Other investigations showed a weak inhibitory effect of this hormone on basal glucose output [6–10]. A more pronounced, antagonistic effect of insulin was observed after a glucagon-induced increase of glucose output [11–14]. Apparently an insulin-dependent net glucose uptake has never been demonstrated in isolated perfused liver. Even the perfusion of unphysiologically high glucose concentrations failed to produce an increase of glucose uptake after the addition of insulin [14]. The reason for this lack of an insulin effect is not clear [15]. A defect in isolated liver preparations has been proposed [16].

The possibility was considered that the regulation of hepatic glucose metabolism might not be a function of the absolute concentration of this substrate but of a glucose concentration gradient between the portal vein and the hepatic artery. It was the aim of this investigation to examine this hypothesis. Therefore, a new method was established for the simultaneous perfusion of rat liver via the hepatic artery and the portal vein. It was found that a glucose gradient with a higher concentration in the portal vein is indeed an important signal for the insulin-dependent increase of net glucose uptake. By this mechanism the liver might be able to differentiate between endogenous and exogenous glucose.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of reagent grade and from commercial sources. Enzymes and bovine serum

albumin were purchased from Boehringer (D-6800 Mannheim). Insulin was from Serva (D-6900 Heidelberg).

2.2. Animals

Male Wistar rats (150–250 g) were obtained from Winkelmann (D-4791 Borcheln). At least one week before the experiment they were subjected to a 12 h day-night rhythm with free access to food (standard diet 1320 of Altromin, D-4937 Lage). All experiments were started at 9 a.m. The animals were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body wt).

2.3. Liver perfusion

The liver was perfused in situ without recirculation in a 37°C cabinet. At the beginning of the preparation open ligatures were positioned around 5 different blood vessels: (a) the aorta cranial of the coeliac trunc, (b) the mesenteric artery, (c) the gastroduodenal artery, (d) the splenic vein and (e) the inferior caval vein (fig.1). The oesophagus and the hepatogastric ligaments were cut in order to loosen the tight connection between liver and stomach. The 'usual' monovascular perfusion system via the portal vein was established by cannulating this vessel and cutting the inferior caval vein open. At this stage liver perfusion via the portal vein was started at 10 mmHg with an erythrocyte-free Krebs-Henseleit-bicarbonate medium. Then the ligatures around the mesenteric artery (b), the splenic vein (d) and the gastroduodenal artery (c) were closed (fig.1). The aorta was cannulated at the ramification of the aorta into the common iliac arteries; the tip of the canula was pushed forward to the origin of the mesenteric artery. At this stage the additional perfusion via the artery was begun at 120 mmHg with the medium described above. After closing the ligature around the aorta cranial of the coeliac trunc (a) the inferior caval vein was cannulated and the canula of the aorta and the caval vein were sewed with the quadratus lumborum muscle and the sigmoidal section of the colon in order to avoid bleedings out of these two vessels. At this stage the perfusion medium was changed to an erythrocyte-containing medium. Thus a bivascular perfusion system was established with a 'low-pressure' com-

ponent via the portal vein (about 65–80% of flow) and a 'high-pressure' component via the hepatic artery (about 20–35% of flow).

The basic perfusion medium was a Krebs-Henseleit buffer containing 30% (v/v) bovine erythrocytes, 9.5 mM glucose, 2 mM lactate, 0.2 mM pyruvate, 2 mM alanine, 1 mM glutamine, 2 mM ornithine, 0.2 mM ammonium chloride and 0.5% bovine serum albumin. In some experiments the glucose concentration was lowered to 6 mM as indicated in the figure legends. The medium was equilibrated with a gas mixture of 13% (v/v) O₂, 5% (v/v) CO₂ and 82% (v/v) N₂ mimicking arterial tensions via both vessels. The effluent medium was collected after an initial period of 30 min and cooled on ice. Insulin was infused reaching final concentrations in the perfusion medium as indicated in the figures. Metabolites were measured with standard enzymatic techniques, glucose with glucose dehydrogenase and lactate with lactate dehydrogenase [17].

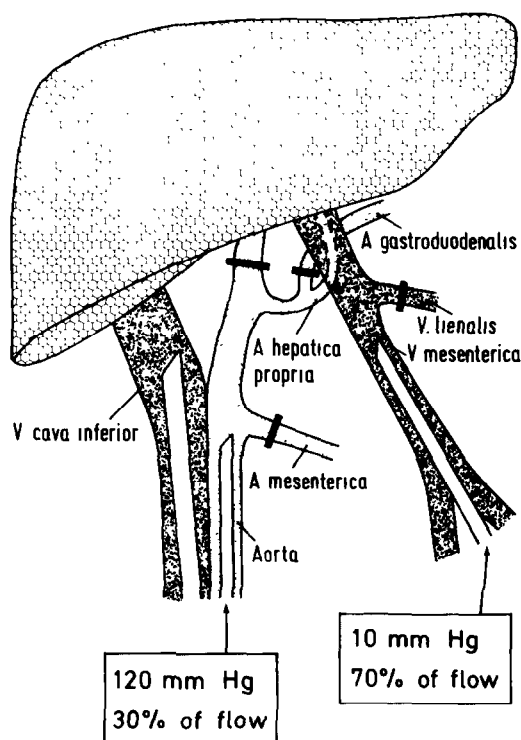


Fig.1. Scheme of bivascular liver perfusion via the hepatic artery and the portal vein. For details see text.

3. RESULTS

3.1. Bivascular liver perfusion

Rat livers were perfused in situ without recirculation via the portal vein and the hepatic artery. Throughout the whole experiment the distribution of flow was held constant with a physiological portion of 20–35% for the hepatic artery. Physiological conditions were also simulated by establishing a high-pressure inflow (~120 mmHg) via the hepatic artery and a low-pressure inflow via the portal vein (~10 mmHg). Although the hepatic artery was not directly cannulated in this preparation, bleedings via branching arteries were reliably prevented by ligating all these vessels. Alternatively in some preliminary experiments the common hepatic artery was directly cannulated with a polyethylene tube. With this procedure only the gastroduodenal artery has to be ligated. Nevertheless, the direct cannulation has the important disadvantage of being more difficult and time consuming because the hepatic artery is a nearly rectangular branching from the abdominal aorta and

thus damaging of this vessel could only be prevented by very careful handling of the polyethylene tube. Therefore, this direct cannulation seems to be only an alternative for rats with higher weight and correspondingly larger vessels.

3.2. Influence of a portal-arterial glucose gradient on insulin-dependent glucose uptake

As controls rat livers were perfused monovascularly only via the portal vein. The media contained substrates favouring glycogen synthesis [18]. In the presence of 10 mM glucose insulin provoked only a small decrease of glucose output (fig.2a). Similar experiments were performed with the bivascular perfusion technique. When 9.5 mM glucose was offered via both vessels, insulin added via both routes caused only a slight decrease of glucose output analogous to the effect in the monovascular perfusion (fig.2b). When, however, a glucose gradient was established with a high glucose concentration in the portal vein (9.5 mM) and a low concentration in the hepatic artery (6 mM), insulin infused via both vessels

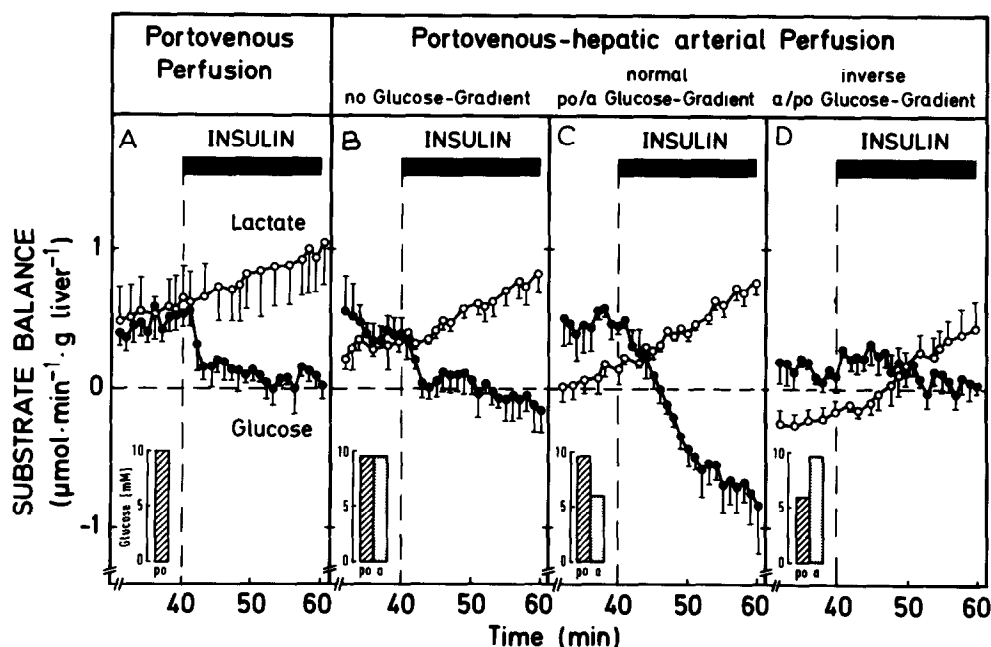


Fig.2. Glucose and lactate balance in mono- and bivascular perfusions of rat liver. Glucose was supplied via the artery (a) and the portal vein (po) in the concentrations indicated in the lower left inset in each panel. Insulin was infused when indicated at 100 nM via the portal vein (A) or via both the portal vein and the hepatic artery (B–D). Values are means \pm SE of 3 separate perfusions each.

caused a switch from glucose output to uptake (fig.2c). This continuous increase of glucose uptake was not completely finished even 20 min after the start of insulin infusion.

When an inverse gradient of glucose with 6 mM in the portal vein and 9.5 mM in the hepatic artery was established, insulin did not cause a significant alteration of the glucose balance (fig.2d). Even the insulin-dependent reduction of glucose output as seen in perfusions without a glucose gradient (fig.2a,b) was not observed in the presence of an inverse glucose gradient. Therefore an insulin-dependent increase of glucose uptake was only demonstrable in isolated liver perfusion when a glucose concentration gradient between the portal vein and the hepatic artery (lower glucose levels) was established.

Neither the conversion of the experimental system to a bivascular perfusion nor the establishment of a glucose gradient between the portal vein and the hepatic artery influenced significantly the insulin-dependent alteration of the lactate balance (fig.2a-d).

3.3. *Influence of a portal-arterial insulin gradient on insulin-dependent glucose uptake*

It was examined further whether not only a glucose gradient but also an insulin gradient could act as a signal for the insulin-dependent alteration of glucose metabolism. When 9.5 mM glucose was supplied via both the portal vein and the hepatic artery, insulin infused with a gradient (the portal vein: 150 nM; hepatic artery: 30 nM) caused a switch from glucose output to uptake (not shown) similar though less pronounced as when a glucose gradient was offered. However, when both a glucose and an insulin gradient (only 2-fold; portal vein: 140 nM; hepatic artery: 70 nM) were established, no further increase of glucose uptake was observed (not shown).

4. DISCUSSION

4.1. *Arterial-portal interactions*

The isolated liver perfused monovascularly via the portal vein has been for many years and still is an important experimental model for the study of liver metabolism. The model is eo ipso clearly unphysiological in one respect, it excludes possible contributions from the hepatic artery. This may be

acceptable at least for metabolic studies as long as one finds it difficult to envisage how a hepatocyte should differentiate in the mixed sinusoidal blood whether a substrate or hormone was delivered via the portal vein or via the artery. It might be less acceptable also for metabolic investigations if one considers the possibility that different signals reaching the hepatocytes might be generated by substrates and hormones in the hepatic arterial and portal vascular tree. Indeed, for the regulation of liver hemodynamics arterial-portal interactions appear to be well established [19], e.g. intraportal isoproterenol is without direct effect on portal flow but increases hepatic arterial flow almost to the same degree as intraarterial isoproterenol [20]. Such arterial-portal interactions may be important also for the regulation of liver metabolism. Therefore a perfusion system was established which allows the simultaneous perfusion of rat liver via the hepatic artery and the portal vein with physiological flow rates and pressures (fig.1). It could be shown with this system that a portovenous-arterial concentration gradient of glucose is an important signal for the insulin-dependent increase of glucose uptake (fig.2). Thus not only hemodynamic but also metabolic processes appear to be dependent on arterial-portovenous interactions.

4.2. *Mechanism of sensing arterial-portal concentration gradients*

The mechanism by which an arterial-portal gradient of glucose or any other substrate could be sensed and transmitted to the hepatocytes is totally unknown. A speculation may be based on the finding that afferent impulses from hypothetical hepatic glucose receptors ascend through afferent fibre of the vagus [21,22] and transmit glucose-related signals from the liver to the autonomic centre of the hypothalamus, which in turn may control via efferent fibres hepatic glucose metabolism. Thus it is conceivable that an intrahepatic nervous network 'measures' the glucose gradient and generates the appropriate signal within the liver to render the hepatocytes insulin-sensitive.

4.3. *Physiological significance of the portal-arterial glucose concentration gradient*

It is a widely held view that the liver in the postabsorptive situation supplies glucose if re-

quired by extrahepatic organs and that in the absorptive phase it removes a substantial part of the excess glucose provided by a carbohydrate-rich meal. Thus normally the liver takes up only exogenous glucose. A straightforward way to differentiate between endogenous and exogenous glucose would be to sense a portal-arterial glucose concentration gradient, since the portal concentration will only be higher than the arterial one if glucose is absorbed from the gut at a high rate. The possible significance of portal-arterial glucose gradients for hepatic glucose uptake has also been indicated by preliminary reports on in vivo studies with dogs [23].

Recently, the view that the liver takes up glucose, e.g. mainly for glycogen synthesis, has been challenged; data have been presented showing that lactate rather than intact glucose is a major precursor of liver glycogen [24]. It was assumed that lactate was formed by the skeletal muscles, which however could not be corroborated by in vivo measurements [25,26]. An obvious way out of the dilemma is provided by the model of metabolic zonation [27–29] which postulates that the periportal hepatocytes should synthesize glycogen from C₃ substrates and that the perivenous cells should form it from glucose and at the same time convert glucose to lactate. Thus glucose uptake remains an essential function of the liver.

The insulin-induced shift from hepatic glucose output to uptake, based on a portal-arterial glucose gradient, could be an important mechanism for the maintenance of glucose homeostasis.

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